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REAPPRAISAL OF PROCTODONE INVOLVEMENT IN THE HORMONAL REGULATION OF LARVAL DIAPAUSE

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Recent findings of juvenile hormone (JH) involvement in larval diapause may form the basis of a revised neuroendocrine theory of larval diapause regulation. It has now been independently confirmed that the mature larval diapause of the southwestern corn borer, *Diatraea grandiosella* Dyar, and the rice stem borer, *Chilo suppressalis* Walker, is initiated and maintained by JH (Chippendale and Yin, 1973; Yin and Chippendale, 1973; Yagi and Fukaya, 1974). These diapause larvae retain actively secreting corpora allata, and the circulating JH enforces inactivity in the brain-ecdysial gland system. Neural and/or neurosecretory axons originating in the brain probably regulate the secretory activity of the corpora allata during diapause. We are now trying to determine exactly how the brain controls the corpus allatum.

As a first step, we have reexamined whether an abdominal hormone, proctodone, controls the activity of the cerebral neurosecretory system of diapause larvae. The ileal epithelium of the european corn borer, *Ostrinia nubilalis* (Hübner), has been named as an endocrine center which responds to photoperiodic signals by the rhythmical secretion of proctodone thereby controlling cerebral neurosecretory activity and diapause development and prepupal morphogenesis (Beck, 1964, 1968; Beck and Alexander, 1964a, b; Beck, Colvin and Swinton, 1965; Beck Shane, and Colvin, 1965; Alexander and Fahrenbach, 1969). The proctodone-cerebral neurosecretory interaction was considered to be the first step of diapause development with proctodone involvement ceasing once the cerebral neurosecretory system was fully activated to secrete ecdysiotropin. Briefly, this hypothesis states that the onset and regulation of diapause is regulated by the phase relationships of an 8 hour proctodone secretory rhythm (phase set by the onset of darkness) and an 8 hour cerebral neurosecretory rhythm (phase set by the onset of illumination). Proctodone is said to activate the neurosecretory system under long days (16L:8D, diapause averting) when the two rhythms are in phase, but not under short days (12L:12D, diapause inducing) when the two rhythms are out of phase. This two oscillator model was used to explain the photoperiodic regulation of the larval diapause of *O. nubilalis* (Beck, 1968).

Although this proctodone function has received wide publicity, the existence of the hormone has never been independently confirmed. McLeod, Graham and Hannay (1969) have cast doubt on its validity and Beck (1974a) did not include it in his new photoperiodic determination model of insect development and diapause. We therefore undertook the present study in an attempt to resolve whether such a hormone originates in the larval ileum of *D. grandiosella* and *O. nubilalis* as a regulator of diapause development and prepupal morphogenesis.

Materials and Methods

Test larvae

Nondiapause and diapause larvae (6th instar) of *D. grandiosella* and diapause larvae (5th instar) of *O. nubilalis* were used for the experiments. These crambid and pyrausid moths are both now assigned to the family Pyralidae and enter diapause at the end of their last larval instars. Laboratory-reared *D. grandiosella* were obtained from our stock culture which is maintained on an artificial diet (Chippendale, 1975). Nondiapause larvae were reared at 30° C 12L:12D, reached maturity around 14 days, remained spotted, and pupated shortly thereafter. Diapause larvae were reared at 23° C 12L:12D and reached maturity around 40 days. Prediapause spotted larvae ecdyse into immaculate larvae with pigment-free integument to mark the onset of diapause (Chippendale and Reddy, 1972, 1973). Field-collected diapause southwestern corn borers were also used. These specimens weighed about 300 mg each and were collected from their overwintering site in the root crowns of corn plants from the delta region of southeastern Missouri in December 1973 (mid-diapause larvae) and September 1974 (newly-diapaused larvae). Newly-diapaused larvae of the european corn borer, weighing about 100 mg each, were collected from corn stalks in southeastern Missouri in September 1974, and central Missouri in October 1974. Unlike *D. grandiosella, O. nubilalis* does not have any morphological characteristics to mark the onset of its larval diapause. The non-feeding diapause larvae of both species were held individually in sterilized 23 × 85 mm glass vials containing moist paper strips. The number and origin of the larvae used in the experiments are listed with the results.

Abdominal ligature and nerve cord severance experiments

The effect of a silk thread ligature placed between the 6th and 7th abdominal segments on the rate of diapause termination was examined (Beck and Alexander, 1964b). This ligature removed the ileum, the proposed site of proctodone secretion, from the anterior compartment. Larvae ligatured between the 9th and 10th abdominal segments served as controls. The ventral nerve cord was also severed between the 6th and 7th segments to isolate the terminal abdominal ganglion from the nervous system. The nerve cord was ruptured with a pair of fine forceps and the operation caused little extraneous tissue damage. The following diapause larvae, including untreated controls, ligatured test and control groups, and in two cases nerve cord severed groups, were used: field-collected *D. grandiosella* held at 30° C 24L:OD and 30° C 12L:12D, 60 day old immaculate laboratory-reared *D. grandiosella* held at 30° C 24L:OD, and *O. nubilalis* held at 30° C 15L:9D. An additional untreated control group of *O. nubilalis* was held under a short day, 30° C 12L:12D. These three regimes permitted differing rates of diapause development (Chippendale and Reddy, 1973; Beck and Alexander, 1964b). Larvae were observed daily until all the individuals in the test groups had either died or pupated.

Bioassay of ileal extracts

A bioassay of ileal extracts of *D. grandiosella* was conducted to determine whether they accelerated the pupation rate of diapause larvae (Beck *et al.*, 1965b).

The ileal epithelium was dissected out of mature 14–16 day old nondiapause larvae, stripped of surrounding muscles, rinsed free of hemolymph, and stored in a sterile 0.85% saline solution at − 20° C. Samples were collected shortly after the onset of the photophase because the proctodone hypothesis predicts that the epithelial cells should then be packed with hormone-containing granules when larvae are held under a 12L : 12D photoperiod (Beck *et al.,* 1965a). After being homogenized, the ileal preparation was centrifuged for 20 min at 15,000 g at 0–5° C. The concentration of the resulting supernatant was then adjusted to 0.5, 1.0 or 1.5 ileal equivalents/5 μl saline and immediately injected into the abdomen of field-collected diapause larvae. Larvae which served as solvent controls received 5 μl of the sterile saline solution. The bioassay was conducted as follows: prior to receiving the injection the test larvae were preconditioned at 30° C 12L : 12D until about 5% had pupated. They were then injected, transferred to 25° C 12L : 12D to permit a lower rate of diapause development, and observed for 30 days. If the ileal extract bypassed the environmental regulatory system the test larvae should have pupated more rapidly than those in the control.

Histology of the ileum

The histology of 16 day old nondiapause mature larval and newly-ecdysed pupal ileum was compared using standard procedures. Anterior intestines were dissected out of larvae at the beginning of the photophase and out of white pupae which had molted about 2 hours earlier. They were fixed in aqueous Bouin's solution, embedded in paraplast, and cut into a series of 6 μ thick transverse sections. The sections were stained with a paraldehyde fuchsin solution (Ewen, 1962), and photographed using a Wild M20 system and Panatomic X film.

Fluorescence microscopy of ileal epithelium

The proctodone hypothesis suggests that the hormone is released into the hemolymph at the beginning of the scotophase and approximately 8 hr thereafter in an ultradian rhythmical fashion. This rhythm was established by examining the autofluorescence of fresh ileal epithelium. The autofluorescence was correlated with the presence of paraldehyde fuchsin-positive granules and proctodone activity in the ileal cells (Beck *et al.,* 1965a). We examined the ileal epithelium of mature larvae of both *D. grandiosella* and *O. nubilalis* for both autofluorescence and acridine orange (AO)-induced fluorescence. For autofluorescence observations, ileal epithelium was removed, washed, cut longitudinally, flattened out on microscope slides, and immersed in 0.85% saline solution. The epithelium was examined under a Wild M20 fluorescence microscope and photographed using Tri X film. A similar procedure was carried out for AO fluorescence observations, with the exception that the newly dissected ileal epithelia were immersed in an AO in saline solution (0.005%) for 30 min. Observations were made according to the proposed empty and full cell stages of the proctodone secretory rhythm for larvae held at 30° C 12L : 12D. Preparations were set up about 2 hr from the end of the photophase when most of the ileal epithelia should have exhibited autofluorescence, at the beginning of the scotophase, and 4 hr into the photophase when the autofluorescence in many of the preparations should have been at least partially quenched.

Electron microscopy of ileal epithelium

Ileal cells of mature 16 day old nondiapause larvae which displayed bright autofluorescence were fixed in 3% glutaraldehyde in Sorenson's buffer (pH 7.2) for 3 to 4 hours. They were post-fixed in 2% buffered osmium tetroxide for 3 to 4 hr at 4° C and then dehydrated and embedded in Spurr's epoxy resin. After only 30 min of glutaraldehyde fixation and prior to post-fixation, some cells were incubated in a medium to detect acid phosphatase activity using β-glycerophosphate as the substrate (Brunk and Ericsson, 1972). Controls were run by adding sodium fluoride (10 mM) as an inhibitor to the incubation medium. Sections (< 1000 Å) were cut with a Reichert ultramicrotome, placed on 200 mesh copper grids, and stained with a 2% alcoholic uranyl acetate for 25 min and, for the non-incubated tissues, alkaline lead citrate for 5 min at room temperature (Venable and Coggeshall, 1965). Electron micrographs were taken using an RCA-EMU-3G instrument operated at 100 KV.

Results

Larval response to abdominal ligatures and nerve severance

Figure 1 illustrates the response of diapause larvae of *D. grandiosella* and *O. nubilalis* to a ligature applied between the 6th and 7th abdominal segments. If, as is suggested by the proctodone hypothesis, the ileum is the hormonal source for terminating diapause, the test larvae should remain in diapause. Figure 1A shows that untreated field-collected larvae of *D. grandiosella* held at 30° C 24L : OD reached 50% pupation in 22 days whereas larvae in the ligatured test and control groups did not even attain this mark. Only 32% of the former and 20% of the latter pupated, and the remaining larvae died during the 38 days of observation. Similar results were obtained when field larvae were exposed to 30° C 12L : 12D (Fig. 1B). In this case the untreated controls reached 50% pupation in 39 days, and only 12% of the ligatured test larvae and 8% of the ligatured control larvae pupated. The remaining larvae died within the 68 days' observation period. Severing the ventral nerve cord between the 6th and 7th abdominal segments resulted in a higher pupation rate than in the ligatured test and control groups, but still caused a high mortality rate. The results show that 68 days after treatment 38% of the larvae had pupated, leaving the remainder dead. Figure 1C illustrates the effects of the abdominal ligature on laboratory-reared *D. grandiosella* exposed to a 30° C 24L : OD regime. As before, only 16% of the ligatured test larvae and 2.5% of the ligatured control larvae survived to pupate, whereas 50% of the untreated controls had pupated in 35 days. We must therefore conclude that these results do not confirm the existence of any factor in the 7–9th abdominal segments whose exclusion from the anterior compartment would prevent pupation. The high mortality rate in the ligatured groups was probably due to severe stress caused by the partitioning of the hemocoel. However, more ligatured test than ligatured control larvae pupated in all 3 experimental situations.

Figure 1D summarizes the results of the abdominal ligature and nerve cord severance experiment conducted on diapause larvae of *O. nubilalis* held at 15L : 9D. The results show that 50% of the untreated larvae pupated in 50 days. Larvae held in another untreated control group at 30° C 12L : 12D (n = 40, not

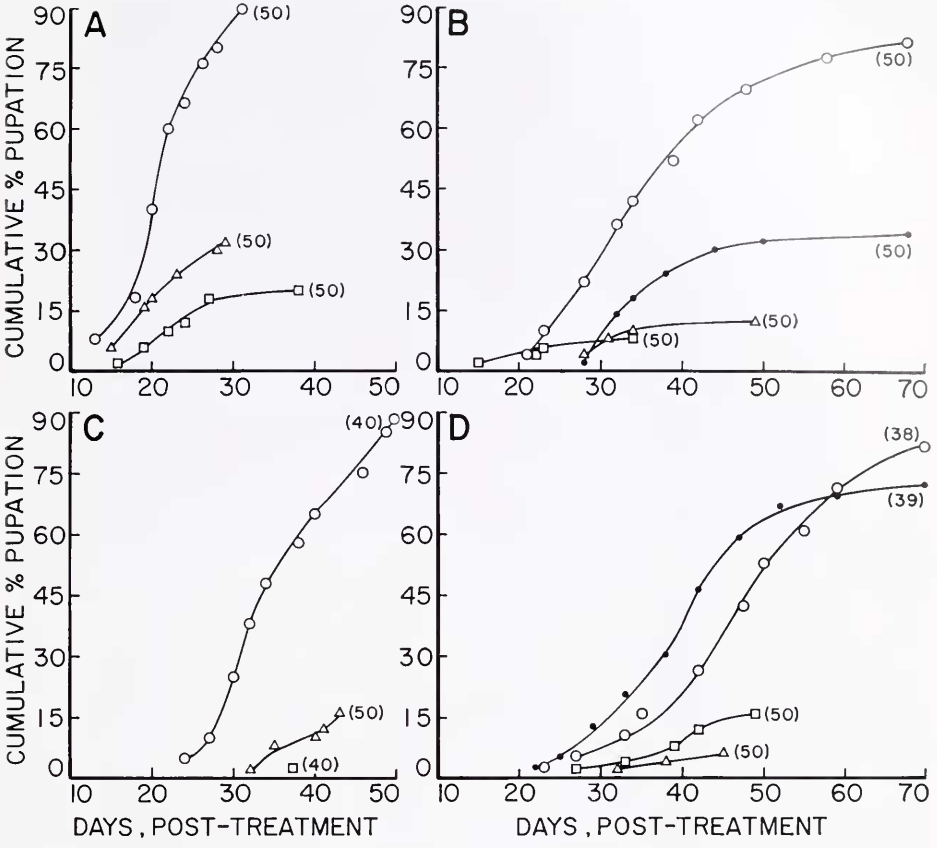

FIGURE 1. Effect of abdominal ligatures and nerve cord severance on the rate of diapause development of *Diatraea grandiosella* and *Ostrinia nubilalis*. A) Field-collected *D. grandiosella* larvae (Dec. 1973) maintained in a 30° C 24L:OD regime; B) field-collected *D. grandiosella* larvae (Dec. 1973) maintained in a 30° C 12L:12D regime; C) laboratory-reared *D. grandiosella* larvae (60 days old) maintained in a 30° C 24L:OD regime; and D) field-collected *O. nubilalis* larvae (Oct. 1974) maintained in a 30° C 15L:9D regime. Open circles represent untreated control; open triangles represent larvae ligatured between 6th and 7th abdominal segment (test); open squares represent larvae ligatured between 9th and 10th abdominal segment (control); closed circles represent larvae whose nerve cord was severed between 6th and 7th abdominal segment. Parenthetic numbers refer to the initial number of larvae in each group.

illustrated) did not pupate during 70 days of observation, thereby confirming the pronounced effect of photoperiod on diapause development of these Missouri-collected borers (McLeod and Beck, 1963). In contrast, many of the ligatured test and control larvae died within 70 days, and only 6% of the former and 15% of the latter pupated. These data again show that the ligature is often lethal to the larvae. No significant differences were observed between the ligatured test and control groups. We therefore did not obtain any evidence that larval diapause of *O. nubilalis* is prolonged by a ligature applied between the 6th and 7th abdominal

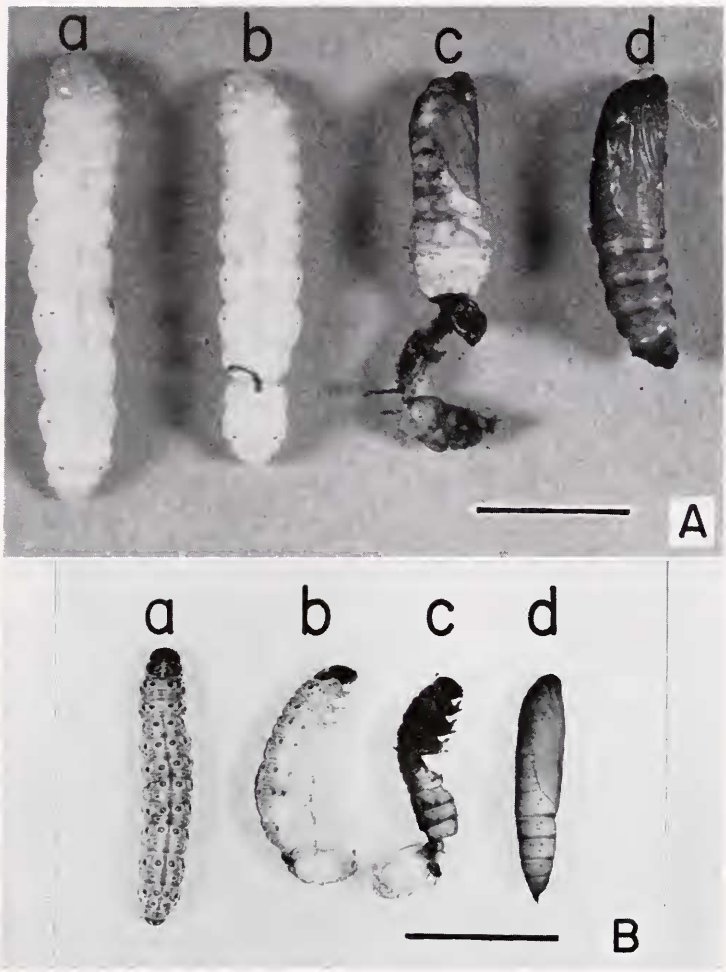

FIGURE 2. Larvae and pupae of the southwestern (A) and european (B) corn borers illustrating the abdominal ligature experiment. (a) Shows normal diapause larva; (b) shows diapause larva ligatured between the 6th and 7th abdominal segments; (c) shows pupa formed following the abdominal ligature; and (d) shows normal pupa. Scale bars equal 1 cm.

segments. Larvae whose nerve cord was severed survived well and pupated at a slightly higher rate than the untreated controls. This treatment resulted in a lower mortality in the european than in the southwestern corn borer (*cf.* Fig. 1B). While the significance of this result is not yet clear, it is possible that isolating the terminal abdominal ganglion from the central nervous system frees the brain from an inhibitory neural influence which normally is involved in sustaining diapause.

Figure 2 illustrates the four classes of *D. grandiosella* and *O. nubilalis* observed in the ligature experiment and also serves to compare the larval and pupal forms of the two species. Mature southwestern corn borers are about 3 times as large

TABLE I

Effect of an ileal extract from mature nondiapause larvae on diapause development of the southwestern corn borer.

Larvae* (number)	Regime	Treatment‡	30 days post-treatment		
			Larvae (%)	Pupae (%)	Mortality (%)
65	30° C 12L:12D	Untreated control	55	22	23
65	25° C 12L:12D	Untreated control	91	5	4
25	25° C 12L:12D	Solvent control	80	4	16
35	25° C 12L:12D	0.5 Ileal equiv./L	84	11	5
40	25° C 12L:12D	1.0 Ileal equiv./L	85	5	10
36	25° C 12L:12D	1.5 Ileal equiv./L	83	3	14

* Diapause larvae collected from the field in September 1974 were used. They were preconditioned for 38 days at 30° C 12L:12D until about 5% had pupated, before the remaining larvae were divided into the experimental groups.

‡ Each test larva received an injection of 5 μl of an ileal saline extract prepared from mature nondiapause larvae (14–16 days old).

as the equivalent european corn borers. The immaculate diapause southwestern corn borer is illustrated. The spotted nondiapause and prediapause morphs differ only in the presence of pigmented integumental pinacula.

Response of diapause larvae to an ileal extract

A proctodone bioassay which had previously given positive results for *O. nubilalis* was performed on *D. grandiosella* (Beck *et al.*, 1965b). This bioassay was crucial in the formulation of the original proctodone hypothesis since it provided the only direct evidence for the existence of an abdominal hormone. Table I shows that the injection of ileal extracts did not accelerate diapause development. Since 22% of the larvae in the untreated control held at 30° C 12L:12D pupated compared with only 5% of those transferred to 25° C 12L:12D, the 25° C regime was stringent enough to uncover any proctodone effect. The results show, however, that 30 days after treatment no significant differences were found between the number of larvae and pupae in the 2 control and 3 test groups. The few pupal molts recorded at 25° C occurred periodically over the 30 day observation period. The injection of an ileal extract did not bypass the normal environmental programming of diapause development, and therefore no proctodone effect was detected.

Histology of the larval and pupal ileum

The larval proctodeum of both species is divided into the pyloric valve and ileum (anterior intestine) and the rectum. The histology of the ileum of *O. nubilalis* larvae has already been described (Beck *et al.*, 1965b). We examined the comparative histology of the mature larval and newly-ecdysed pupal ileum of *D. grandiosella* (Fig. 3). This borer's mature larval ileum is similar to that of *O. nubilalis* and is made up of large squamous epithelial cells which are present

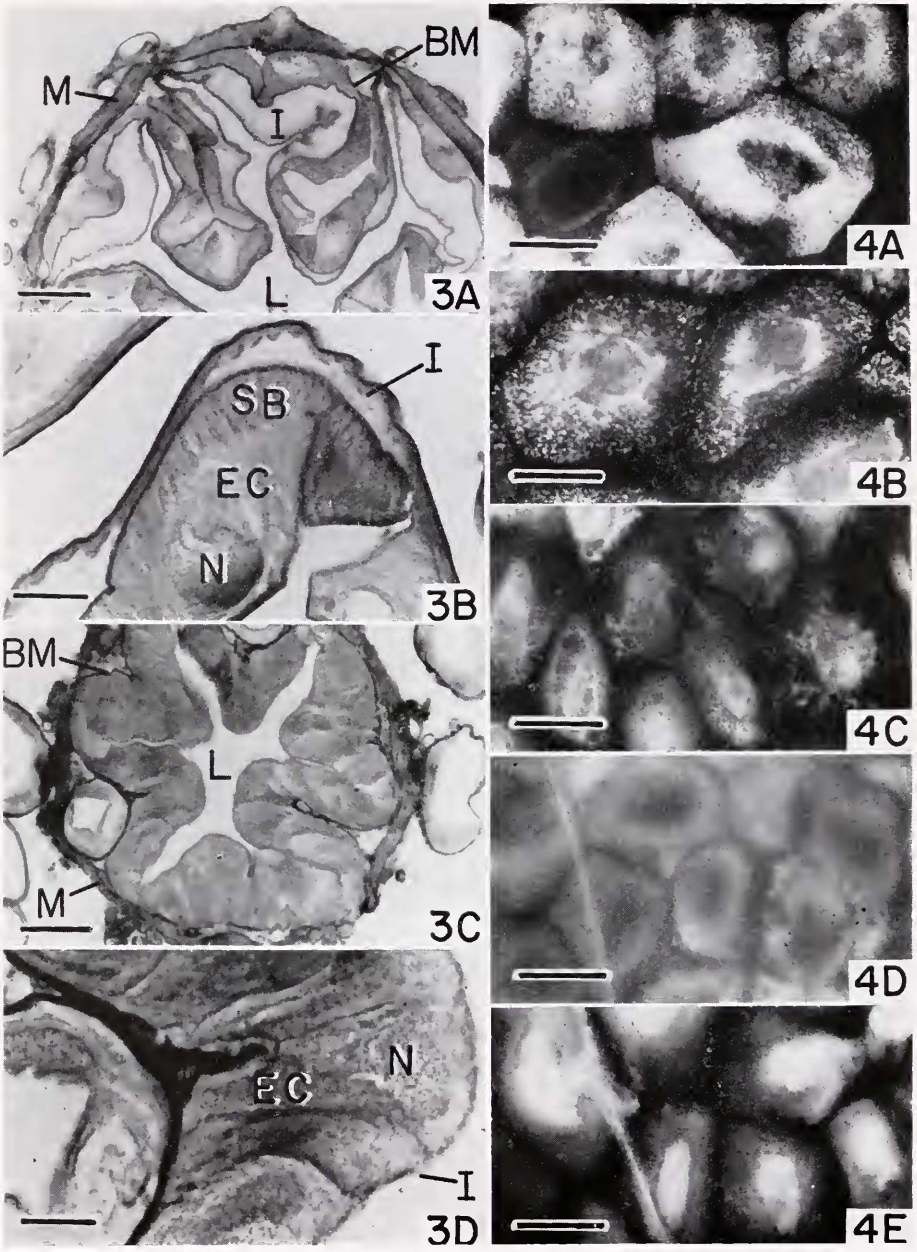

FIGURE 3. Histological sections of the ileum from mature larvae (A, B) and newly molted pupae (C, D) of the southwestern corn borer. Symbols used are: BM, basement membrane; EC, epithelial cell; I, intima; L, lumen; M, circular muscle; N, nucleus; SB, striated border. Scale bars equal 40 μ (A and C), 10 μ (B and D).

FIGURE 4. Autofluorescence and acridine orange-induced fluorescence in the ileal epithelium of the southwestern and european corn borer. A, B, show ileum from late stage mature

in 6 longitudinal infoldings in the empty gut (Fig. 3A). A cuticular intima, distinct nuclei and striated borders in the epithelial cells, basement membrane, and circular musculature are clearly visible (Fig. 3B). Although these sections were prepared from an ileum which exhibited autofluorescence, no distinct paraldehyde-fuchsin positive granules were detected in the cytoplasm. The presence of autofluorescence and paraldehyde-fuchsin positive granules has been correlated with the retention of proctodone activity in the ileum of *O. nubilalis* (Hassemer and Beck, 1969).

In contrast to the larval ileum, the pupal ileum of *D. grandiosella* is much smaller and has a different histological structure (Fig. 3C, D). The infoldings of the epithelial cells, though still present, were less pronounced. An indistinct intima was present, and the columnar epithelial cells lacked a striated border. Deposits of paraldehyde-fuchsin positive granules were not detected. These findings show that the ileum undergoes substantial changes at the beginning of metamorphosis. It is likely that the proposed secretory function of the mature larval ileum is associated with these cellular changes.

Fluorescence of ileal epithelium

Since an ultradian cycle of autofluorescence in the ileum is believed to correlate with the secretion of proctodone (Beck *et al.*, 1965a), we examined the ileal epithelium of *D. grandiosella* and *O. nubilalis* for the presence of both auto and induced fluorescence (Fig. 4). Figure 4A illustrates autofluorescent particles in the ileal epithelium of nondiapause mature larvae of *D. grandiosella*. Ileal epithelium displaying bright green autofluorescence was much more common in pharate pupae than in mature larvae. Figure 4B shows AO-induced fluorescence in the ileal epithelium of a mature nondiapause larva of *D. grandiosella*. This preparation also displayed autofluorescence before AO treatment and the location of the cytoplasmic AO-positive particles corresponded to the autofluorescent ones (*cf.* Fig. 4A). Following this AO treatment a green fluorescence was induced in the nucleus and an orange one in the cytoplasm indicating that the fluorescent cytoplasmic particles are probably lysosomes (Allison and Young, 1969). Figure 4C illustrates AO-induced fluorescence in the ileal epithelium of an early diapause larva of *O. nubilalis*. The nucleus fluoresced green and the cytoplasmic the orange induced fluorescent particles. We conclude that they are lysosomes Figure 4D shows the absence of autofluorescent material in the ileum of *D. grandiosella* while Figure 4E shows that only fluorescent nuclei are detected in the non-autofluorescent epithelium which had been incubated with AO. These last observations confirm that the green autofluorescent particles correspond with the orange induced fluorescent particles. We conclude that they are lysosomes which are presumably involved in remodeling the ileum at the onset of metamorphosis.

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nondiapause southwestern corn borers displaying autofluorescence (A) and acridine orange-induced fluorescence (B); C, ileum from field-collected diapause european corn borer (Sept. 1974) displaying acridine orange-induced fluorescence; D, E, ileum from mature nondiapause southwestern corn borers showing absence of autofluorescence (D), and acridine orange-induced fluorescence in cell which lacked autofluorescence (E). Scale bars equal 40 μ.

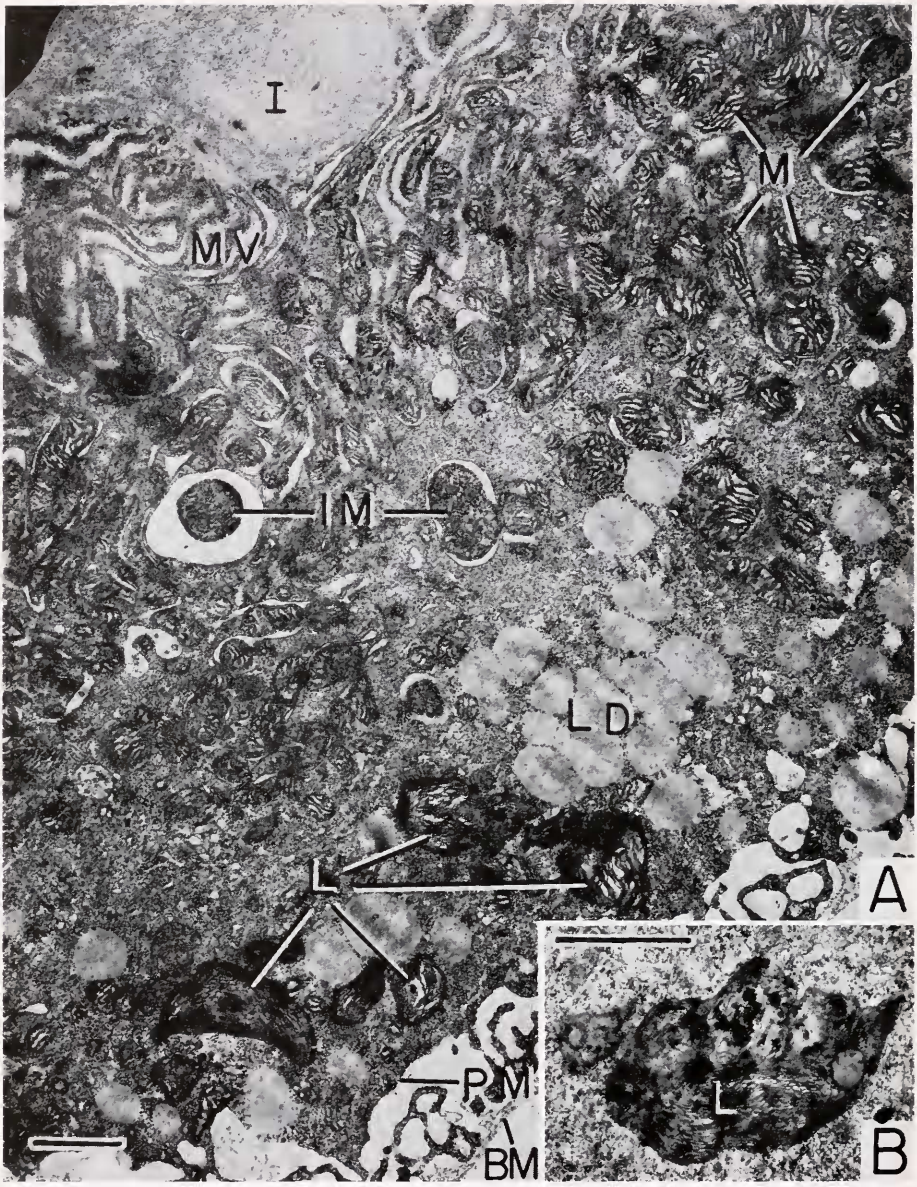

FIGURE 5. Fine structure of an autofluorescent ileal cell of a mature nondiapause southwestern corn borer (A), and an ileal lysosome following a histochemical test for acid phosphatase activity (B). Symbols used are: BM, basement membrane; I, intima; IM, isolated mitochondrion; L, lysosome; LD, lipid droplet; M, mitochondrion; MV, microvilli; PM, plasma membrane. Scale bars equal 1 μ.

Ileal tissue was prepared for fluorescence microscopy at periods when the cells should have been both empty and full of autofluorescent particles (Beck *et al.*,

1965a). Although in both species we observed cells which displayed cytoplasmic fluorescence and others which were completely devoid of fluorescence, we were unable to detect any pattern of fluorescence which corresponded to an eight hour autofluorescence rhythm. However, a rigorous study of the rhythmical appearance and disappearance of the autofluorescence was not undertaken and these observations are based upon ileal preparations from about 50 southwestern and 25 european corn borers. Consequently, while our findings suggest that an underlying rhythmicity regulates the appearance of these fluorescent particles, we did not obtain any convincing evidence to support the existence of an eight hour ultradian rhythm which is phase-set by the onset of darkness.

Ultrastructure of ileal epithelium

The electron microscope was used to examine the cytoplasmic particles of the ileal epithelium of mature southwestern corn borers which had ceased feeding. Figure 5A shows a typical preparation from an autofluorescent ileum of a mature nondiapause larva. From the lumen side the cell contains: cuticular intima; mitochondria, associated with the microvilli of the striated border or within isolation bodies; lipid droplets; lysosomes; infolded plasma membrane; and is bounded by a basement membrane. The identity of the laminated membranous organelles as lysosomes was confirmed following the acid phosphatase procedure (Fig. 5B). No lead deposits were seen in the organelles of cells which had been incubated with the acid phosphatase inhibitor, sodium fluoride. The organelles contained lead precipitates deposited at local sites of acid phosphatase activity and therefore have a hydrolytic function which is characteristic of lysosomes. This finding provides additional evidence to show that lysosomes are present in the ileum prior to the onset of metamorphosis.

Discussion

Our results do not support the hypothesis that the ileal cells of the southwestern and european corn borers produce a hormone which interacts with the cerebral neurosecretory system during diapause or at the onset of metamorphosis. Although detailed results were not presented, a proctodone effect in the regulation of the larval diapause of the pine moth, *Dendrolimus pini,* and the codling moth, *Laspeyresia pomonella,* was also ruled out (Kind, 1968; Peterson and Hanner, 1968).

The proposed rhythmical interaction between proctodone and the cerebral neurosecretory system served as the basis for an ultradian two oscillator model of diapause and development (Beck, 1964). During the past 10 years, however, additional research has led to a revision of this model. A most convincing argument for reappraisal came from an experiment in which $> 50\%$ of european corn borer larvae reared under a noncircadian photoperiod of 16L: 16D entered diapause. Since under this regime the postulated proctodone and cerebral neurosecretory rhythms should have been in phase, growth and development of the borer should have proceeded without the intervention of diapause (Beck, 1974a). The two oscillator model has now been superseded by a developmental determination model which better accounts for our existing knowledge about the photoperiodic control of insect development and diapause (Beck, 1974a, b).

Important questions remain concerning the post-digestive functions of the ileum, and whether any abdominal system exerts a regulatory influence on diapause and development. Our finding that the mature larval ileum contains lysosomes confirms the observations of Hassemer and Beck (1968, 1969) and McLeod *et al.* (1969). We also show that the fluorescent particles are lysosomes, thereby providing an alternative explanation for the "proctodone" phenomenon. Lysosomes are known to autofluoresce (Allison and Young 1969), and frequently to be associated with paraldehyde-fuchsin positive material (De Duve and Wattiaux, 1966). The lysosomes observed in the mature larval ileum are presumably autolysosomes which may discharge enzymes and lytic products into the hemolymph (De Duve and Wattiaux, 1966; Lockshin, 1969). This exocytosis would account for the observed secretory activity of the ileum. Furthermore, histological observations usually show that the lepidopteran pupal ileum contains less cellular material than the larval one (Judy and Gilbert, 1970). The mature larval ileum also appears to function as an osmoregulatory center (Hassemer and Beck, 1971). We observed mitochondria associated with the microvilli of the ileum of *D. grandiosella* larvae, and this system presumably actively transports water and ions even during diapause. Osmoregulation encompassing both transport and secretion may represent the principal function of the ileum in post-feeding larvae.

A neural or neurosecretory involvement of the insect abdomen in endocrine regulation of growth and metamorphosis has not yet been detailed. It is possible that the abdominal ganglia provide some essential input to the larval brain to signal the beginning of metamorphosis. Evidence is beginning to suggest that neural signals originating in the abdomen prime the brain to initiate metamorphosis (Edwards, 1966, 1967; Sehnal and Edwards, 1969; Beck, 1970). We have undertaken a preliminary study of the possible involvement of the abdominal ganglia in regulating the onset of pupation of the southwestern corn borer. Although we have not yet obtained any reproducible effects, we recognize that the timing of any possible neural signals is critical and plan to investigate the system further.

We believe that receptors in the larval head receive the photoperiodic and thermal signals which regulate the onset and termination of a facultative diapause (Geispits, 1957; Williams and Adkisson, 1964; Claret, 1966). This exteroceptive input to the brain may be supplemented and modified by proprioceptive input from the abdomen providing information about size, form, and posture (Edwards, 1967; Nijhout and Williams, 1974). The brain then integrates these afferent signals to institute a diapause or nondiapause program. In the case of a facultative mature larval diapause, the larval brain may continue to activate the corpora allata leading to a continued secretion of JH and the institution and maintenance of diapause (Yin and Chippendale, 1973). Larval diapause is probably terminated when environmental signals received by the brain lead to a change in the efferent signal to the corpora allata, resulting in a decline in the hemolymph JH titer, and the initiation of the larval-pupal molting cycle.

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Summary

1. No evidence was found that the larval ileum of the corn borers, *Diatraea grandiosella* and *Ostrinia nubilalis,* secretes a developmental hormone, "proctodone."

2. An abdominal ligature which isolated the ileum of diapause larvae of both species caused high mortality but did not retard the pupation rate.

3. Extracts prepared from the ileal epithelium of mature nondiapause larvae of *D. grandiosella* were injected into diapause larvae and did not cause premature diapause termination.

4. Marked cytological changes occurred in the ileum at the onset of metamorphosis. At times the ileal epithelium of both species displayed auto and acridine orange-induced fluorescence, characteristic of lysosomes.

5. An electron microscopic examination of the autofluorescent ileal epithelium of *D. grandiosella* revealed organelles which had typical lysosomal features and stained positively for acid phosphatase activity.

6. The secretory activity of the ileum can be accounted for by lysosomal involvement in its metamorphic reorganization, and by its osmoregulatory functions.

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